ATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 02 November 2000 (02.11.00)	
International application No. PCT/US00/03534	**Applicant's or agent's file reference** ERI-113Xq999
International filing date (day/month/year) 11 February 2000 (11.02.00)	**Priority date** (day/month/year) 11 February 1999 (11.02.99)
Applicant YOUNG, Michael, J. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

30 August 2000 (30.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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By
JUN 02 2000
WEINGARTEN, SCHURGIN, GAGNEBIN & HAYES

From the INTERNATIONAL SEARCHING AUTHORITY

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To: GWENDOLYN H. YIP
WEINGARTEN, SCHURGIN, GAGNEBIN & HAYES, LLP
TEN POST OFFICE SQUARE
BOSTON, MA 02109

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

	Date of Mailing *(day/month/year)* 30 MAY 2000
Applicant's or agent's file reference ERI-113XQ999	**FOR FURTHER ACTION** **See paragraphs 1 and 4 below**
International application No. PCT/US00/03534	International filing date *(day/month/year)* 11 FEBRUARY 2000
Applicant THE SCHEPENS EYE RESEARCH INSTITUTE, INC.	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID NIKODEM Telephone No. (703) 308-0196

3613 5

PATENT COOPERATION TREATY

PCT

REC'D 07 AUG 2001

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ERI-113XQ999	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/03534	International filing date *(day/month/year)* 11 FEBRUARY 2000	Priority date *(day/month/year)* 11 FEBRUARY 1999

International Patent Classification (IPC) or national classification and IPC
IPC(7): A61K 48/00; C12N 15/85; A01N 63/00 and US Cl.: 424/93.21, 93.7, 93.1; 435/405, 325

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MAY 0 8 2002

TECH CENTER 1600/2900

Applicant
THE SCHEPENS EYE RESEARCH INSTITUTE, INC.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☒ Certain observations on the international application

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GROUP 1600

Date of submission of the demand	Date of completion of this report
30 AUGUST 2000	29 JUNE 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence Fox Anne-Marie Baker, Ph.D. Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998) *

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/03534

I. Basis of the report

1. With regard to the elements of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-42, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the claims:
pages 43-46, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the drawings:
pages 1-4, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	10, 14, 15, and 17-25	YES
	Claims	1-9, 11-13 and 16	NO
Inventive Step (IS)	Claims	None	YES
	Claims	1-25	NO
Industrial Applicability (IA)	Claims	1-25	YES
	Claims	None	NO

2. citations and explanations (Rule 70.7)

Claims 1-9, 13, and 16 lack novelty under PCT Article 33(2) as being anticipated by TAKASHI et al. The reference teaches a method of treating dystrophic neural tissue by introducing adult-derived neural progenitor cells into the developing retina. Said neural tissue is hippocampus-derived. The reference anticipates the claimed invention.

Claims 1, 3, 5, 6, 9, 11, and 12 lack novelty under PCT Article 33(2) as being anticipated by MARTINEZ-SERRANO et al. The reference teaches a method of treating dystrophic neural tissue by introducing adult-derived neural progenitor cells into the neural septum of an adult brain. The reference anticipates the claimed invention.

Claims 1-25 lack an inventive step under PCT Article 33(3) as being obvious over either TAKAHASHI et al. or MARTINEZ-SERRANO et al. in view of WEISS et al. WEISS et al. teaches the use of a variety of trophic factors to treat neural cells in culture prior to transfer to a host, for ex vivo gene therapy and tissue grafts. The purpose of this treatment is to further neural cell growth in culture prior to transfer. It would have been obvious for one of ordinary skill in the art to combine the teachings of WEISS et al. with those of the previously discussed references to aid in culturing of neural progenitor cells to treat dystrophic neural tissue.

---------- NEW CITATIONS ----------

US 5,851,832 A (WEISS et al.) 22 December 1998, see disclosure.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-25 are objected to under PCT Rule 66.2(a)(iii) as containing the following defects in the form or contents thereof: the claims are drawn to a method of treatment of humans, specifically claim 10. The claims are not enabled by the disclosure. The disclosure states treatment of mouse by said method for a specific vision problem. However, this is the only disclosed treatment. No other neural or optical dystrophic conditions are treated. It is unpredictable in the art as to whether or not the claimed treatment method will function for any other disease or disease state. A variety of factors need to be taken into consideration for the delivery and elicitation of a therapeutic effect by a pharmaceutical composition, including: formulation, method of delivery, site of delivery, composition uptake, composition half-life, and composition concentration and efficacy. Undue experimentation would have been required for one skilled in the art to identify and test alll the disclosed trophic factors for ex vivo treatment of cells in the treatment of all dystrophic neural tissue. Thus, only those pharmaceutical compositions identified and enabled in the disclosure carry patentable.

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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60/119,642 11 February 1999 (11.02.99) US

(71) Applicants *(for all designated States except US)*: THE SCHEPENS EYE RESEARCH INSTITUTE, INC. [US/US]; 20 Staniford Street, Boston, MA 02114 (US). THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10280 North Torrey Pines Road, La Jolla, CA 92186-5800 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: YOUNG, Michael, J. [US/US]; 1002 The Heights, Gloucester, MA 01930 (US). GAGE, Fred, H. [US/US]; 6668 Caminito Hermitage, La Jolla, CA 92037 (US). RAY, Jasodhara [US/US]; 4184 Corte de la Siena, San Diego, CA 92130 (US). WHITELEY, Simon, J. [GB/US]; 48 Teele Street, No. 1, Arlington, MA 02174 (US). KLASSEN, Henry [US/US]; 206 Opal Avenue, Newport Beach, CA 92662 (US).

(74) Agents: YIP, Gwendolyn, H. et al.; Weingarten, Schurgin, Gagnebin & Hayes LLP, Ten Post Office Square, Boston, MA 02109 (US).

(81) Designated States: AU, BR, CA, JP, MX, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: INTEGRATION OF TRANSPLANTED NEURAL PROGENITOR CELLS INTO NEURAL TISSUE OF IMMATURE AND MATURE DYSTROPHIC RECIPIENTS

(57) Abstract

The present invention is directed to methods of repairing dystrophic, differentiated neural tissue, such as a damaged or diseased retina or optic nerve, in humans and other animals. In particular, the invention relates to introduction of adult-derived neural progenitor cells into a dystrophic neural tissue site of an animal recipient, including an adult (mature) animal, whether xenogeneic, allogeneic, or syngeneic. These adult-derived, neural progenitor cells can functionally and morphologically integrate into both mature and immature, dystrophic neural tissue.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
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EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03534

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 48/00; C12N 15/85; A01N63/00

US CL : 424/93.21, 93.7, 93.1; 435/405, 325

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.21, 93.1, 93.7; 435/405, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN (medline, biosis, caplus, scisearch, embase), WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTINEZ-SERRANO et al. Immortalized neural progenitor cells for CNS gene transfer and repair. Trends in Neuroscicnce. November 1997, Vol. 20, No. 11, pages 530-538, see abstract and page 530, paragraph 4.	1, 3, 4-15, 18-25.
X	SNYDER et al. Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. Nature. 23 March 1995, Vol. 374, page 367-370, see abstract and page 367, paragraphs 1-5.	1,3,4-15,18-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 13 APRIL 2000	Date of mailing of the international search report 30 MAY 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID NIKODEM Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03534

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKAHASHI et al. Widespread integration and survival of adult-derived neural progenitor cells in the developing optic retina. Molecular and Cellular Neuroscience. December 1998, Vol. 12, pages 340-348, see abstract and page 346, paragraph 2.	2,4-15,16-2 5
X	MARTINEZ-SERRANO et al. CNS-derived neural progenitor cells for gene transfer of nerve growth factor to the adult rat brain: complete rescue of axotomized cholinergic neurons after transplantation into the septum. The Journal of Neuroscience. August 1995, Vol. 15, No. 8, pages 5668-5680, see abstract and page 5668, paragraph 1-2.	1, 3, 4-15,18-25
Y	DURING et al. Towards gene therapy for the central nervous system (review). Molecular Medicine Today. November 1998, Vol. 4, No. 11, pages 485-493, see page 485, paragraphs 3-6.	1-25.

TITLE OF THE INVENTION

Integration Of Transplanted Neural Progenitor Cells Into Neural Tissue Of Immature And Mature Dystrophic Recipients

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/119,642, filed on February 11, 1999, the whole of which is hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

Proliferative cells present in the hippocampus of adult rodent have been isolated, cultured, and transplanted to various sites within the central nervous system (CNS). These cells are capable of differentiating into neurons when grafted to sites where neurogenesis is known to occur (Suhonen 1996, Shihabuddin 1997, Gage 1995). However, prior attempts to use transplanted neurons to repopulate areas of pathological cell loss within the CNS of adult mammals have largely failed, because donor neural cells tend not to integrate with host cells. For instance, attempts to transplant neurons into the eye have not demonstrated morphological integration with the host retina (del Cerro 1992, Silverman 1992, Aramant 1994, Berson & Jacobiec 1999).

As part of the central nervous system, both developmentally and phenotypically, the retina shares the recalcitrance of brain and spinal cord with respect to functional repair. This is unfortunate because, among heritable conditions alone, over 100 examples of

diseases exist that involve the loss of retinal neurons (Bird, 1995; Simunovic and Moore, 1998).

One attempted strategy for replacing diseased retinal neurons has been to transplant retinal tissue from healthy donors to the retina of the diseased host (Gouras et al., 1994; Silverman and Hughes, 1989). While the results of such studies have been encouraging in terms of graft survival, the problem of morphological and functional integration between graft and host has remained daunting. The graft-host interface is often well demarcated histologically, with ultrastructural studies revealing the presence of a dense glial scar across which few neurites are seen to cross (Ivert et al., 1998).

Thus, prior art findings have not provided a viable solution to neural degenerative disorders, particularly in adult animals.

BRIEF SUMMARY OF THE INVENTION

The present invention is directed to methods of treating dystrophic neural tissue, particularly damaged or diseased, differentiated neural tissue, in humans and other animals. It is shown that neural progenitor cells can functionally and morphologically migrate and integrate into mature and immature neural tissue. In particular, disclosed is the first successful, stable morphological integration of neural progenitor cells, e.g., adult hippocampal progenitor cells (AHPCs), into the neural tissue of animals of various ages, including immature, nondystrophic retina of syngeneic recipients (e.g., Fischer rat-derived AHPCs into immature retina of Fischer rats), and notably, diseased adult retina in allogeneic recipients (e.g., Fischer rat-derived AHPCs into dystrophic Royal College Surgeon (RCS) rats). Surprisingly, AHPCs have also been found to integrate

succesfully into a xenogeneic recipient: e.g., rat AHPCs into the retina of dystrophic rd-1 mice.

Thus, the invention encompasses methods of repairing, replacing, augmenting, or rescuing damaged or diseased, differentiated neural tissue, by introducing adult-derived neural progenitor cells into a human or other animal recipient, whether the recipient is syngeneic (of the same species and genetic strain), allogeneic (of the same species but a different strain), or xenogeneic (of a different species) to the donor. In particular, the method comprises introducing neural progenitor cells derived from a healthy donor into dystrophic neural tissue of an animal recipient, including an adult or a young animal. One embodiment of the invention encompasses repopulating or rescuing a dystrophic retina or optic nerve with neurons, by introducing neural progenitor cells, e.g., AHPCs, derived from an adult donor animal, into the dystrophic eyes of an animal recipient.

The neural progenitor cells may be introduced into dystrophic neural tissue by placement within a recipient's central nervous system, an eye, an optic nerve, or vitreous. The recipient can be either an immature (young) or immature (adult) animal.

Advantageously, the neural progenitor cells are derived from adult brain tissue, such as the hippocampus or the ventricular zone. Neural progenitor cells are preferably clonally derived. The neural progenitor cells may, prior to introduction into a dystrophic neural tissue site, have been cultured in vitro in a culture medium comprising at least one trophic factor selected from the group consisting of: a neural growth factor; a neurotrophin; a mitogen; a cytokine; a growth factor; a hormone; and a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a-f depict the localization of grafted AHPCs to specific retinal layers in recipient rats of different ages (4 weeks (a-d); 10 weeks (e), and 18 weeks (f));

Figures 2a-i depict confocal images of expression of neuronal markers by grafted AHPCs in animal grafted at: 4 weeks, examined 4 weeks after grafting (a-c); at 10 weeks, examined 4 weeks after grafting (d-f): at 16 weeks, examined 1 week after grafting (g-I);

Figures 3a-h depict confocal images of grafted cells treated with anti-synaptophysin/Cy3 (red) antibody in animals grafted at 4 weeks, and examined 4 weeks after grafting; and

Figures 4a-c present confocal images of GFP+ neurites projecting, via the host optic fiber layer, into the optic nerve head 4 weeks after grafting (a+b).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to successful transplantation of neural progenitor cells into dystrophic neural tissue. In application, the invention encompasses a method of treating dystrophic neural tissue, comprising introducing neural progenitor cells derived from an adult animal donor into dystrophic neural tissue in an animal recipient, e.g., by grafting or applying adult progenitor cells into tissue affected by the disorder.

The recipient may be an young (immature) animal or an adult (mature) animal. The neural progenitor cell donor and recipient may be of different species (xenogeneic). Exemplary donor-recipient pairs include, but are not limited, to: a donor rat and a recipient mouse; a donor mouse and a recipient rat; a donor pig and a recipient human. The donor and recipient may be

of the same species (e.g., human-to-human, rat-to-rat, mouse-to-mouse), and be allogeneic (of different strains, i.e., have different histocompatibility genes) or syngeneic (of the same strain, i.e., having identical histocompatibility genes).

Examples of dystrophic neural tissue that can be treated by the invention include the central nervous system (CNS) and neural tissue of the eye, particularly the retina or optic nerve. Thus, in one embodiment, the invention encompasses a method of repopulating or rescuing a dystrophic retina with neural cells, comprising introducing neural progenitor cells derived from an adult donor (e.g., AHPCs) into dystrophic neural tissue of an animal recipient. The method is particularly useful for treating dystrophic retinal tissue caused by an optic neuropathy, e.g., glaucoma.

As used herein, the term "dystrophic neural tissue" encompasses damaged, injured, or diseased neural tissue, which neutral tissue includes differentiated neural tissue. Thus the present invention provides methods for treating a neuronal or neural disorder or neural injury. A "neuronal disorder" or "neural disorder" is any disorder or disease that involves the nervous system. One type of neuronal disorder is a neurodegenerative disorder. Neurodegenerative disorders include but are not limited to: (1) diseases of central motor systems including degenerative conditions affecting the basal ganglia (e.g., Huntington's disease, Wilson's disease, Striatonigral degeneration, corticobasal ganglionic degeneration, Tourettes syndrome, Parkinson's disease, progressive supranuclear palsy, progressive bulbar palsy, familial spastic paraplegia, spinomuscular atrophy, ALS and variants thereof, dentatorubral atrophy, olivo-pontocerebellar atrophy, paraneoplastic cerebellar degeneration, cerebral angiopathy (both

hereditary and sporadic)); (2) diseases affecting sensory neurons (e.g., Friedreich's ataxia, diabetes, peripheral neuropathy, retinal neuronal degeneration); (3) diseases of limbic and cortical systems (e.g., s cerebral amyloidosis, Pick's atrophy, Retts syndrome; (4) neurodegenerative pathologies involving multiple neuronal systems and/or brainstem (e.g., Alzheimer's disease, AIDS-related dementia, Leigh's disease, diffuse Lewy body disease, epilepsy, Multiple system atrophy, Guillain-Barre syndrome, lysosomal storage disorders such as lipofuscinosis, late-degenerative stages of Down's syndrome, Alper's disease, vertigo as result of CNS degeneration; (5) pathologies arising with aging and chronic alcohol or drug abuse (e.g., with alcoholism the degeneration of neurons in locus oeruleus, cerebellum, cholinergic basal forebrain; with aging degeneration of cerebellar neurons and conical neurons leading to cognitive and motor impairments; and with chronic amphetamine abuse degeneration of basal ganglia neurons leading to motor impairments; and (6) pathological changes resulting from focal trauma such as stroke, focal ischemia, vascular insufficiency, hypoxic-ischemic encephalopathy, hyperglycemia, hypoglycemia or direct trauma.

The presence of a neuronal or neurodegenerative disorder or injury may be indicated by subjective symptoms, such as pain, change in sensation including decreased sensation, muscle weakness, coordination problems, imbalance, neurasthenia, malaise, decreased reaction times, tremors, confusion, poor memory, uncontrollable movement, lack of affect, obsessive/compulsive behavior, aphasia, agnosia, visual neglect, etc. Frequently, objective indicia, or signs observable by a physician or a health care provider, overlap with subjective indicia. Examples of objective

indicia include the physician's observation of signs such as decreased reaction time, muscle fasciculations, tremors, rigidity, spasticity, muscle weakness, poor coordination, disorientation, dysphasia, dysarthria, and imbalance. Additionally, objective signs can include laboratory parameters, such as the assessment of neural tissue loss and function by Positron Emission Tomography (PET) or functional Magnetic Resonance Imaging MRI), blood tests, biopsies and electrical studies such as electromyographic data.

"Treating" dystrophic neural tissue is intended to encompass repairing, replacing, augmenting, rescuing, or repopulating the diseased or damaged neural tissue, or otherwise compensating for the dystrophic condition of the neural tissue.

"Introduction" of neural progenitor cells into dystrophic neural tissue (e.g., a damaged or diseased retina or optic nerve), may be accomplished by any means known in the medical arts, including but not limited to grafting and injection. It should be understood that such means of introducing the neural progenitor cells also encompass placing, injecting or grafting them into a site separate and/or apart from the diseased or damaged neural tissue site, since the neural progenitor cells are capable of migrating to and integrating into that dystrophic site. For example, dystrophic retinal or optic nerve tissue can be treated by placing neural progenitor cells into the vitreous of the eye.

The neural progenitor cells used in the invention are derived from a healthy adult animal donor, and may come from brain tissue, such as the hippocampus or ventricular zone. Advantageously, adult hippocampal progenitor cells (AHPC) may be used, particularly clonally derived AHPCs cultured in vitro under proliferative conditions.

As used herein, the term "progenitor cell" refers to cells which have the ability to differentiate, including stem cells and progenitor cells. In contrast to undifferentiated cells, differentiated cells have a clearly defined morphology that identifies it as a member of a defined histological type. The cell can be a mammalian cell. In one embodiment, the mammalian cell is a rodent cell. In another embodiment, the cell is a primate cell, such as a human cell. Progenitor cells employed herein refer to both undifferentiated cells whose lineal descendants differentiate along the appropriate pathway to produce a fully differentiated phenotype, as well as founder cells of embryonic or other cell lineage, which are undifferentiated cells displaying high proliferative potential, generating a wide variety of differentiated progeny including the principal phenotypes of the tissue, possessing the capacity for self-renewal and retaining their multi-lineage potential over time (Gage et al. (1995) Annu. Rev. Neurosci. 18:159-192, each herein incorporated by reference). All differentiated cells have, by definition, a progenitor cell type. For example, "neural progenitor cells" such as neuroblasts are progenitors for neurons and germ cells for gamete cells. Additionally, it is readily appreciated that progenitor cells do not differentiate into only one type of cell. For example, neural progenitor cells give rise primarily to neurons, however, such cells can also rise to astrocytes, glial cells and oligodendrocytes. Those of skill in the art will readily recognize the associated progenitor cells for differentiated cells. Stem cells are capable of dividing to produce two daughter cell types with different fates: one is another stem cell identical to the mother cell, and the other is a lineage progenitor cell which will divide to produce more

differentiated cells. In adult mammals, stem cells occur in most tissue systems. For example, the bone marrow gives rise to all blood cells and muscle.

The therapeutic benefit of the invention can be evaluated or assessed by any of a number of subjective or objective factors indicating a response of the condition being treated. Such indices include measures of increased neural or neuronal proliferation or more normal function of surviving brain areas. In addition, macroscopic methods of evaluating the effects of the invention can be used which may be invasive or noninvasive. Further examples of evidence of a therapeutic benefit include clinical evaluations of cognitive functions including object identification, increased performance speed of defined tasks as compared to pretreatment performance speeds, and nerve conduction velocity studies.

In another aspect of the invention, the neural progenitor cells have preferably been cultured in vitro in a culture medium comprising at least one trophic factor, or even combinations of such factors. As used herein, the term "trophic factor" refers to compounds with trophic actions that promote and/or control proliferation, differentiation, migration, survival and/or death (e.g., apoptosis) of their target cells. Such factors include cytokines, neurotrophins, growth factors, mitogens, co-factors, and the like, including epidermal growth factor, fibroblast growth factor, platelet-derived growth factor, insulin-like growth factors, ciliary neurotrophic factor and related molecules, glial-derived growth factor and related molecules, schwanoma-derived growth factor, glial growth factor, stiatal-derived neuronotrophic factor,

platelet-derived growth factor, hepatocyte growth factor, scatter factor (HGF-SF), transforming growth factor-beta and related molecules, neurotransmitters, and hormones. Those of ordinary skill in the art will recognize additional trophic factors that can be employed in the present invention (see, e.g., Aebischer et al. Neurotrophic Factors (Handbook of Experimental Pharmacology, Vol 134) (Springer Verlag, 1998); Meyers, R.A. Encyclopedia of Molecular Biology and Molecular Medicine: Denaturation of DNA - Growth Factors (VCH Pub, 1996); Meager & Robinson, Growth Factors : Essential Data (John Wiley and Sons, 1999); McKay & Brown, Growth Factors and Receptors: A Practical Approach (Oxford University Press, 1998); Leroith & Bondy, Growth Factors and Cytokines in Health and Disease, Vol 1A and 1B : A Multi-Volume Treatise (JAI Pr, 1996); Lenfant et al., Growth Factors of the Vascular and Nervous Systems: Functional Characterization and Biotechnology: International Symposium on Biotechnology of Grow (S. Karger Publishing, 1992).

"Trophic factors" have a broad range of biological activities and their activity and specificity may be achieved by cooperation with other factors. Although trophic factors are generally active at extremely low concentrations, high concentrations of mitogen together with high cell density are often required to induce proliferation of multipotent neural progenitor cell populations. For example, growth factors for early progenitors may be useful for enhancing the viability of progenitor cells as well as treating disorders by renewal of mature cells from the progenitor cell pool.

Preferred trophic factors contemplated for use in the present invention are mitogenic growth factors, like fibroblast growth factor-2 (FGF-2) (Gage, F.H., et al., 1995, Proc. Natl Acad. Sci. USA 92:11879-11883) and

epidermal growth factor (EGF) (Lois, C., and Alvarez-Buylla, A., 1993, Proc. Natl. Acad. Sci. USA 90(5):2074-2077), which induce proliferation and/or propogation of progenitor cells, e.g., neural progenitor cells isolated from the brain. Studies from single cells in culture demonstrate that FGF-2 (Gritti, A., et al., 1996, J. Neurosci. 16:1091-1100) and EGF (Reynolds, B.A., and Weiss, S., 1996, Develop. Biol. 175:1-13) are mitogens for multipotent neural stem cells and likely cooperate with other trophic factors (Cattaneo, E., and McKay, R., 1990, Nature 347:762-765; Stemple, D.L., and Anderson, D.J., 1992, Cell 71:973-985), some of which are yet unknown (Davis, A.A., and Temple, S., 1994, Nature 372:263-266; Temple, S., 1989, Nature 340:471-473; Kilpatrick, T.J., and Bartlett, P.F., 1993, Neuron 10:255-265; Palmer, T.D., et al., 1997, Mol. Cell. Neurosci. 8:389-404) to achieve specificity.

As used herein, the neural progenitor cells can be cultivated in the presence of a trophic factor, or combinations of trophic factors. For example, these cells can be cultivated in medium having "neurotrophins" (or "neurotrophic factor") that promote the survival and functional activity of nerve or glial cells, including a factor that enhances neural differentiation, induces neural proliferation, influences synaptic functions, and/or promotes the survival of neurons that are normally destined to die, during different phases of the development of the central and peripheral nervous system. Exemplary neurotrophins include, for example, ciliary neurotrophic factor (CNF), nerve growth factor (NGF), fibroblast growth factor (FGF), brain-derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3), glia derived neurotrophic factor (GDNF), and the like. Such factors are characterized by their trophic actions, their expression patterns in the brain, and molecular

aspects of their receptors and intracellular signaling pathways. Neurotropic factors that have been identified include NT-4 , NT-5 , NT-6 , NT-7 , ciliary neuronotrophic factor (CNTF), Glial cell line-derived neurotrophic factor (GDNF), and Purpurin. Neuron-specific enolase (NSE) has been found to be a neuronal survival factor. Other factors possessing a broader spectrum of functions, which have neurotrophic activities but are not normally classified as neurotrophins, also are contemplated for use in the invention. These factors include epithelial growth factor (EGF), heparin-binding neurite-promoting factor (HBNF), IGF-2, a-FGF and b-FGF , PDGF , neuron-specific enolase (NSE), and Activin A. Other factors have been identified which specifically influence neuronal differentiation and influence transmitter phenotypes without affecting neuronal survival. Although the intracerebral administration of FGF-2 has been shown to stimulate neurogenesis in the adult rat SVZ, FGF-2 alone in the adult rat hippocampus has a limited effect on the proliferation of neural stem/progenitor cells (Kuhn et al. (1997); Wagner et al. (1999) each herein incorporated by reference).

In a preferred embodiment of the present, the present invention employs FGF and FGF-like factors, including a-FGF, b-FGF such as FGF-2, FGF-4, FGF-6, and the like. A particularly advantageous medium for culturing neural progenitor cells comprises one of the following: fibroblast growth factor (FGF) alone (particularly basic FGF or FGF-2), FGF plus epidermal growth factor (EGF), or FGF plus EGF plus heparin, which is mitogenic.

For example, the neural progenitor cells may be derived by the following steps:

(a) isolating fresh neural progenitor cells from an adult donor animal;

(b) culturing said freshly isolated neural progenitor cells on a polyornithene/laminin-coated substrate, in a culture medium containing at least one trophic factor, selected from the group consisting of FGF-2 alone, FGF-2 plus EGF, and FGF-2 plus EGF plus heparin;

(c) incorporating an identifying, genetic marker into said cultured progenitor cells; and

(d) cloning individual hippocampal progenitor cell lines from the cultured cells resulting from step (c).

Additionally, the methods of the invention can further comprise, prior to introducing the neural progenitor cells into a recipient, confirming the lineage potential of each clone of clonally derived adult hippocampal progenitor cells by inducing a sample of said clonally derived hippocampal progenitor cells to differentiate in "conditioned medium", a term of art referring to medium or supernatant removed from cultures of living cells and then filtered.

The invention also encompasses a kit for generating neural progenitor cell lines derived from an adult donor animal, comprising the following:

(a) a polyornithine/laminin-coated substrate (e.g., a coated tissue culture vessel);

(b) a culture medium containing at least one trophic factor selected from the group consisting of or a combination thereof;

(c) a vector comprising an identifying genetic marker, for incorporation into hippocampal progenitor cells (HPC) isolated from an adult animal, upon culture of those cells (e.g., green fluorescent protein (GFP));

(d) an article of manufacture comprising instructions for cloning at least one hippocampal progenitor cell line from hippocampal progenitor cells isolated from an adult donor animal.

In yet another embodiment of the present invention, there are provided methods for treating neuronal disorders, the method comprising increasing the level of adult progenitor cells in dystrophic tissue. Progenitor cells can be grafted into the tissue ex vivo (by cultivating the cells in vitro) or can be cultivated in vivo. As contemplated herein, the progenitor cells can be native to the dystrophic tissue but propagated and/or proliferated by the administration of trophic factors in vivo or in vitro. In a preferred embodiment of the present invention, the progenitor cells can be propagated or proliferated in vitro, and incorporated or re-incorporated into the dystrophic tissue. Alternatively, trophic factors can be administered to the dystrophic tissue to increase the level of native or transplanted progenitor cells.

The invention is further described by way of the following, non-limiting examples.

Example I: Intra-Species, Allogeneic Retinal Transplant

Clonally derived, adult rat hippocampal progenitor cells (AHPCs), genetically modified to express green fluorescent protein (GFP), were injected into the eyes of dystrophic RCS rats of various ages. When subsequently examined, the retinas of these animals exhibited widespread migration of green fluorescent protein-expressing (GFP^+) donor cells into all layers of the host retina. The transplanted cells survived for at least 2 months post-grafting, without provoking a

prominent immune response. Furthermore, GFP$^+$ cells aligned themselves with the existing cytoarchitecture and exhibited extensive arborization in configurations appropriate for retinal neurons. Similar results were obtained with both immature and visually mature, recipient animals. These results indicate that the dystrophic retina can be substantially repopulated by using a line of adult-derived, neural progenitor cells from an allogeneic donor, and that these cells can be functionally integrated, since they arborize extensively within the host neuropil.

It has recently been shown that proliferative cells present in the adult rodent hippocampus (Altman and Das, 1965) can be isolated (Palmer et al., 1997), cultured (Gage et al., 1995 and 1998), and transplanted into various sites within the CNS, where they can differentiate into neurons (Gage et al., 1995; Shihabuddin et al., 1997; Suhonen et al., 1996). The present data indicate that transplanted, adult hippocampal progenitor cells (AHPCs) provide a more effective source of donor material for retinal transplantation. Specifically, the data show that these cells can migrate into the dystrophic retina of adult Royal College of Surgeons (RCS) rats, an extensively studied model of retinal degeneration (LaVail et al., 1975; Matthes and LaVail, 1989; Villegas et al., 1998). That is, transplanted AHPC cells can migrate into, and differentiate within, the mature retina during the active phase of neuronal degeneration.

Methodology

Donor cell line: Hippocampal progenitor cells were clonally derived from adult Fischer 344 rats, genetically modified to express the modified jellyfish (Aequorea victoria) enhanced green fluorescent protein

GFP (eGFP). In some cases, the cells were pulsed prior to transplantation with BrdU (e.g., 5 µm, 2 days, or more preferably, 50 ng/ml, 3 pulses over 3 days). Specifically, AHPCs were cultured and differentiated as follows. Primary adult hippocampal progenitor cultures were prepared from hippocampal tissues of 3-month-old female Fisher 344 rats as previously described (Gage et al. 1995a). Dissociated cells were cultured on polyornithine/laminin coated dishes using a mixture of DMEM/Ham' F-12 (1:1) supplemented with N2 (Gibco) and 20 ng / ml FGF-2 (human recombinant, prepared in E. coli, kindly provided by A. Baird). Individual cells were genetically marked using replication-defective retroviral vectors expressing GFP from a tetracycline-regulatable, minimal human cytomegalovirus immediate early promoter fused to a tet-operator (NIT-GFP). Cloned cultures were derived from bulk-injected cultures. Each AHPC clone carried a neomycin phosphotransferase gene (neo) and the enhanced green fluorescence protein (GFP) gene. To confirm the lineage potential of each clone prior to grafting, AHPCs were induced to differentiate in 4-well chamber slides at a cell density of 2,500 cells per cm^2 by withdrawal of FGF-2 and treatment for 14 days in DMEM/F12 + N2, supplemented with 0.5 µM all-trans retinoic acid and 0.5% fetal bovine serum. These conditions were previously shown to favor the differentiation of neurons, astrocytes, and oligodendrocytes in a single well (Palmer et al. 1997). AHPCs were prepared for grafting in the following manner. Cultured AHPCs were harvested with trypsin, washed with high glucose Dulbecco's PBS (D-PBS, Gibco), and suspended at a density of 100,000 cells per µl in D-PBS containing 20 ng of FGF-2 per ml.

Recipient animals and transplantation: At the age of 3-28 days, pigmented dystrophic RCS rats (graft duration before sacrifice: 1 week, n=22; 4 weeks n=41; 10 weeks, n=6; 18 weeks, n=4; 36 weeks, n=9), and albino dystrophic rats (graft duration: 1 week, n=8; 10 weeks, n=6) received injections of AHPCs into the vitreous or subretinal space under general (Ketamine/xylazine) and topical (proparacaine) anesthesia. Injections were performed under direct observation using coaxial illumination via binocular surgical microscope (Möller) through a dilated pupil (topical tropicamide 1%). The injections were made via a beveled glass micropipette (outer diameter of 1 mm) connected to a 50-µl Hamilton microsyringe via PE tubing. The sharp tip of the micropipette allowed direct entry to the vitreous cavity through a self-sealing wound, the entry point being just vitread to the corneo-scleral junction. This approach to the vitreous avoided trauma to the ciliary body and lens, but necessarily resulted in focal perforation of the intervening uvea and peripheral retina. A total of 50,000-100,000 cells in 1-2 µl of DMEM/F12 media were injected. As a control, cells that were freeze-thawed 3 times (from -70 °C) were also injected (n=6).

Tissue preparation and histology: Recipient animals were killed with an overdose of sodium pentobarbitol at 1, 2, 4, 8, and 16 weeks post-transplantation. The eyes were removed and immersion-fixed with 4% paraformaldehyde for 4 hours at 4 °C. The anterior segment and lens were then removed, and the posterior segment cryoprotected in 30% sucrose/PBS overnight at 4 °C, followed by embedding in OCT and subsequent sectioning at 7-14 µm on a cryostat. Sections were processed for haematoxilin and eosin, anti-BrdU (1:400), anti-synaptophysin (1:200) and anti-GFP (1:500), anti-calbindin (1:1000), anti-rhodopsin (1:200), or anti-NF-200 (1:40), anti-MAP-5 (1:500), anti-GFAP (1:200), followed by reaction with Cy3-conjugated secondary antibodies (1:150), thus allowing co-localization of these markers with the endogenous GFP expressed in transplanted AHPCs. Confocal microscopy was carried out on a subset of material that was of particular interest.

Morphology: Retinae containing high numbers of grafted cells were analyzed to determine their laminar localization at 4 and 8 weeks post-transplantation. Age at time of transplantation (1, 4, and 10 weeks) was also compared. A total of nine 50 µm wide regions of sectioned retina were analyzed for each animal, chosen so that both central and peripheral regions were included.

Results

Clonally derived AHPCs from adult Fischer 344 rats, which were genetically modified to express green fluorescent protein (GFP) and also labeled with BrdU in some cases, were transplanted into both immature (3 days postnatal, P3) and mature (4-36 weeks postnatal), dystrophic eyes of RCS rats. Following transplantation, donor-derived cells were found to maintain high levels of GFP expression. GFP+ cells were clearly evident under FITC illumination and were verified to be of graft origin based upon anti-GFP immunoreactivity, anti-BrdU immunoreactivity, as well as constitutive GFP expression (data not shown). The GFP^+ cells were quite striking in appearance and were easily distinguished from autofluorescence of host photoreceptor outer segments in the recipient, based on intensity, morphology, location, and spectral specificity. Subsequent identification of donor-derived cells was therefore based on GFP fluorescence alone, obviating the need for prelabeling with BrdU or the use of anti-GFP antibodies.

At 4 weeks following the injection of AHPCs into the vitreous of immature and mature dystrophic RCS rats, at least 50% of the injected cells survived and maintained high levels of GFP expression in approximately 80% of the 1, 4, 10, and 18 week old recipients, while no surviving cells were found in the 36 week old recipients.

Already at one week post-grafting, grafted AHPCs could be seen adhering to the vitreal surface of the graft recipient or host's eye, migrating into the host retina, and taking up residence within the cellular retinal laminae of the host, including the outer nuclear layer. In some cases, grafted cells were seen in the host photoreceptor layer, and when examined with anti-BrdU, were found to be double labeled with GFP and BrdU, confirming the cells' derivation from the transplanted AHPCs. No evidence of viable donor cells, or host GFP expression, was seen following injection of freeze-thawed GFP^+ AHPCs (negative control), confirming observations reported in Takahashi 1998, incorporated herein by reference.

At subsequent times post-grafting, widespread migration and morphological integration of grafted AHPCs into the host retina was seen. GFP+ cells were found within the retina of 60%, 35%, 48%, and 60% of animals grafted at the ages of 1, 4, 10, and 18 weeks, respectively. At 8 weeks post-grafting, intra-retinal GFP+ cells were found in 80% of the recipients who were 1 week old at the time of grafting, and 50% of those initially 4 weeks old.

TABLE I

Percentage of GFP-expressing cells found in each region of the retina

	1 week old recipient		4 week old recipient		10 week old recipient
Region	4 weeks post-transplant	8 weeks post-transplant	4 weeks post-transplant	8 weeks post-transplant	4 weeks post-transplant
Vitreous	74.4±18.7	15.3±4.1	28.7±7.9	2.2±2.0	48.8±6.7
GCL/IPL	5.8±1.5	7.2±4.2	4.9±1.2	32.3±6.3	7.9±1.7
INL/OPL	4.9±2.4	17.5±3.4	17.0±5.7	5.4±1.6	32.5±3.3*
ONL/SRS	14.9±4.7	60.0±10.6	49.3±9.7	60.1±7.6	10.8±3.2*

GCL/ILP = ganglion cell layer and inner plexiform layer
INL/OPL = inner nuclear layer and outer plexiform layer
ONL/SRS = outer nuclear layer and subretinal space

*N.B. At this time point clearly defined outer plexiform and outer nuclear layers are not present

Table 1 shows the laminar distribution of migrating AHPCs in representative 1, 4, and 10 week old recipients. The majority of grafted cells left the vitreous and entered the retina, where they migrated into the various laminae. Although grafted cells were also found in the ganglion cell and inner nuclear layers, they showed a predilection for the outer retina, particularly the outer nuclear layer, subretinal debris zone and intervening layer of photoreceptor elements (collectively designated "ONL/SRS"). At a later time point (8 weeks post-injection), the number of cells in the ONL/SRS was greater yet. GFP+ cells appeared to gain access to the retina either by direct radial migration through the undamaged vitreal surface or, in greater numbers, by way of the peripheral injection tract with subsequent lateral migration. In the latter case, cells could be found migrating into as much as 60%

of the longitudinal extent of the neuroretina. Regardless of the course taken by the migrating AHPCs, GFP+ cells were found in all layers of the host neuroretina, but not in the retinal pigment epithelium, choroid, or sclera.

Figures 1a-f depict the localization of grafted AHPCs to specific retinal layers in rats of different ages (4 weeks (a-d); 10 weeks (e), and 18 weeks (f)). Cells (green) were grafted into the vitreous of 4 (a-d), 10 (e), and 18 (f) week old rats, and examined 4 weeks later. Retina sections were labeled with anti-synaptophysin/Cy3 antibody (red) to demarcate the synaptic and cellular layers of the host retina, and viewed under FITC and Cy3 fluorescent illumination. The arrow in Fig. 1a indicates cell seen in Fig. 1b at higher power; the arrow in Fig. 1c indicates cell seen in Fig. 1d at higher power (vit, vitreous; gcl, ganglion cell layer; ipl, inner plexiform layer; inl, inner nuclear layer; opl, outer plexiform layer; onl, outer nuclear layer; srs, subretinal debris and degenerating photoreceptor elements).

In Figure 1, numerous GFP+ cells exhibiting neuronal morphologies can be seen. These cells were found in all cellular layers of the host retina, yet tended to respect the plexiform layers (particularly the inner plexiform layer) where they elaborated arbors. Moreover, the configuration of the neuritic processes extended by grafted cells often resembled those of normal retinal neurons: neurites preferentially projected either laterally (i.e. resembling those of horizontal or amacrine cells) or radially (i.e. resembling bipolar cell processes; see Fig. 1b). Whether this reflects intrinsic or extrinsic developmental factors, or is simply a consequence of

restrictions imposed by the local retinal cytoarchitecture remains to be determined.

Additionally, further study of recipient animals treated according to the method of the invention (aged 1 week at time of transplant), has shown that the grafted cells exhibit axonal growth into the optic nerve at or about 8 weeks post-graft.

A number of markers were evaluated to determine whether grafted cells had adopted mature neuronal phenotypes. The results are shown in Figure 2a-i. Figure 2a-i depict confocal images of expression of neuronal markers by grafted AHPCs. Figs. 2a-c are from animals grafted at 4 weeks of age, examined 4 weeks after grafting: constituitive GFP expression (a), anti-calbindin/Cy3 immunoreactivity (b), and merged image (c). The arrows indicate 2 cells co-expressing these labels. Figs. 2d-fare from animals grafted at 10 weeks of age, examined 4 weeks after grafting: constitutive GFP expression (d), MAP-5/Cy3 immunoreactivity (e), merged image (f). The arrows indicate 2 cells co-expressing these labels. Fig. 2g-i are from animals grafted at 16 weeks of age, examined 1 week after grafting: constituitive GFP expression (g), anti-NF-200 /Cy3 immunoreactivity (g), and merged image (i). Arrows indicate 2 cells co-expressing these labels.

A subpopulation of GFP+ cells were found to co-express calbindin, a marker found on some retinal interneurons (Fig. 2a-c), while others co-expressed the neuronal marker MAP-5 (Fig. 2 d-f) or NF-200 (Fig 2 g-I). These results contrast to an earlier report, in which AHPCs grafted into the developing eye of normal animals failed to express neuronal markers (Takahashi 1998). While these markers are not retina-specific, they do show that hippocampal-derived progenitor cells are capable of developing mature neuronal phenotypes

when grafted to a novel site such as the retina. Furthermore, the expression of these markers was regionally appropriate, with calbindin expression confined to transplanted AHPCs in the inner nuclear layer, and NF-200 expression seen predominantly in the ganglion cell layer. Significantly, grafted AHPCs did not show any evidence of GFAP expression or astrocytic morphological development, suggesting a preference for neuronal differentiation in the microenvironment of the degenerating retina.

As GFP+ cells frequently developed elaborate neuronal arbors, the relationship between donor neurites and synaptophysin expression was investigated. Although widely dispersed throughout the retina, the vast majority of synaptophysin seen was localized to the plexiform layers, consistent with host origin. From their positions in the cellular layers, grafted cells frequently extended processes into these layers, apparently in a directed manner.

Figure 3a-h depicts confocal images of grafted cells treated with anti-synaptophysin/Cy3 (red) antibody, which show grafted AHPCs (green) sending processes into the inner plexiform layer (a-d), or the outer plexiform layer (e-h) (grafted at 4 weeks, examined 4 weeks after grafting). In Fig. 3a-b, a cell is shown merged (a), and reconstructed to show entire neuritic arbor (b). In Fig. 3c-h, AHPCs send neurites into the inner plexiform layer (c, higher power in d), and outer plexiform layers (e + g, higher power in f + h, respectively). These processes intermingle with, and appear to contact synaptophysin-positive profiles of the host.

In Figs. 3 a-d, large GFP+ cells are observed to send neuritic processes into the host inner plexiform layer, while Figs. 3 e-h show cells with elaborate

arbors intermingling with the host outer plexiform layer. Figs. 3 a-b provide one example of the way in which the configuration of GFP+ arbors frequently reflects the orientation of the host plexiform layers. One process tracks along the INL/IPL interface while another, originating from an position offset within the ILP, assumes a parallel course in the opposite direction despite the lack of a laminar interface to guide it. Confocal analysis confirmed that large numbers of GFP+ processes come into direct apposition to host synaptophysin + profiles (Fig. 3e-h; 4 weeks post-grafting into 4 week old hosts).

Grafted AHPCs are also capable of extending processes into the host optic nerve. Grafted cells residing in the ganglion cell layer extend neurites with large growth cones that approach, but do not cross, the level of the scleral outlet at 4 weeks post-grafting into 1 week old hosts (Figs. 4a-b). Figures 4a-c show confocal images of GFP+ neurites projecting, via the host optic fiber layer, into the optic nerve head 4 weeks after grafting. These fibers have large growth cones (arrows in Fig. 4a, and in higher power in Fig. 4b), which approach, but do not cross, the scleral outlet (labeled "sc") at 4 weeks post grafting into initially 1 week-old hosts. When animals were examined 8 weeks after grafting, numerous growth cone-tipped processes were found to have entered the optic nerve, extending over 300 µm beyond the scleral outlet (Fig. 4c).

When examined at 8 weeks post-grafting, large numbers of growing neurites were found to cross the scleral outlet, and extend long processes at least 300 µm into the optic nerve (Fig. 4c). The apparent increased density of GFP^+ processes at successive time points indicate that AHPCs continue to develop along a

neuronal-like pathway for at least 8 weeks post-grafting.

No evidence of immunological rejection, decreased cell survival, or decreased gene expression was observed over the course of this study. The range of graft survival and incorporation obtained in different aged hosts (high level of incorporation in animals up to 10 weeks of age at time of transplant, lower level of incorporation seen in 18 week-old recipients, no survival in 36 week-old recipients) suggests that the progressive degeneration occurring in the RCS retina (which begins at 3 weeks of age) contributes to this variability. As the rat retina is fully developed before the end of the 3rd postnatal week, the widespread incorporation seen at 4 and 10 weeks indicates that developmental maturity is not a barrier to the acceptance of AHPCs by the diseased mammalian retina.

Discussion

This study shows that neuronal progenitor cells derived from adult, differentiated neural tissue (e.g., hippocampus), can migrate in large numbers into all layers of the dystrophic neuroretina of mature animals, including, in some cases, the photoreceptor layer.

Following migration, transplanted AHPCs respect the local laminar organization and exhibit a surprising ability to differentiate into neurons with morphological characteristics suggestive of native retinal cell types. The cell processes extended by AHPCs within the retina tend to resemble the neuritic profiles of specific retinal neurons, including sublamina-specific ramifications within the inner plexiform layer suggestive of bipolar and horizontal cells (Dowling, 1970). Furthermore, the presence of distinct bands of

diffuse GFP-derived fluorescence along these subiaminar zones suggests a network of fine terminals within the host neuropil.

These data indicate that neural progenitor cells such as AHPCs are capable of functional integration into the retina of animals up to 10 weeks of age, as well as limited incorporation into 18-week-old recipients, an age when the RCS retina has degenerated severely and other interventions are ineffective. At 36 weeks of age, however, AHPCs not only fail to enter the retina but show very little survival, suggesting the loss of an important trophic influence late in the course of the dystrophy.

Having migrated into the retina from the vitreous, grafted AHPCs disperse within the host tissue rather than remaining adherent to each other, as is typically seen with embryonic neural grafts. After taking up residence, these cells differentiate along neuronal (as opposed to glial) lines and extend processes within the host plexiform layers. Furthermore, the orientation of many of these processes is reminiscent of the arborization pattern of retinal amacrine cells. AHPCs in the ganglion cell layer frequently extend neurites into the optic fiber layer and optic nerve.

More recently, neural progenitor cells have reportedly been found to differentiate into cells of the hematopoietic lineage (Bjornson 1999), suggesting that a hippocampal to retinal fate shift should not be dismissed. Morphologically, AHPC arborizations appear to respond to extrinsic retinal cues in preference to any intrinsic hippocampal developmental programs. Finally, while the finding reported here that graft-derived neurites are intimately associated with host synaptophysin profiles does not conclusively demonstrate synapse formation, another laboratory using this same

AHPC cell line has recently provided electron microscopic evidence of synapse formation in vitro, as well as excitatory post-synaptic potentials (Toda 1999). These results reinforce the conclusion that the neuronal repopulation method achieved here represents morphological and functional integration, rather than simply cellular infiltration or random migration and neurite extension.

Neural progenitor cells such as AHPCs migrate and integrate into neonatal, nondystrophic, syngeneic Fischer rat hosts (see Takahasi 1998, incorporated herein by reference). AHPCs also readily migrate into mechanically injured retina of adult, syngeneic hosts as well as diseased retina of mature, allogeneic RCS rat hosts.

The preceding results are consistent with more recent studies in which stem or progenitor cells seem to respond to the presence of pathology. For instance, neural stem cells grafted to the bloodstream of irradiated mice repopulate the bone marrow (Bjornson 1999), while similar cells grafted to the cerebral ventricles of neonatal shiverer mice replace lost oligodendrocytes (Yandava 1999). Neural progenitor cells clearly possess a high degree of plasticity (Johansson 1999, Flax 1998, Brustle 1998, Morrison 1999) and provide a new tool for studying mechanisms of neural development and degeneration.

The data presented here provide the first definitive evidence for the survival, migration, and neuronal differentiation of a transplanted cell in diseased, mature retina. This study shows that neural progenitor cells can overcome many of the obstacles to neuronal integration present in the mature mammalian central nervous system.

The observations here of widespread morphological integration in an allogeneic situation, also argues for the importance of the specific microenvironment of the host retina in promoting migration and differentiation of grafted precursor cells. The same is expected for other specialized or differentiated neural tissue into which AHPCs are integrated.

The present invention will enable the attainment of the ultimate goal of restorative neuronal transplantation into the eye: introduction of new photoreceptor cells. The data demonstrate that neuroprogenitor cells such as AHPCs are capable of repopulating the outer nuclear layer of the dystrophic retina with cells resembling neurons. The surprising degree of plasticity exhibited by AHPCs transplanted to the diseased eye indicates that using neural progenitor cells to repopulate the eye with photoreceptor cells, seemingly impossible only a few years ago, is now a realistic objective.

One of ordinary skill in the art of neuronal transplantation will appreciate how to practice the present invention and to manipulate AHPCs and other neural progenitor cells to account for such factors as functional capability, host immunological tolerance, and the long-term consequences of grafting (e.g., promoting graft survival and controlling undesired proliferation). The demonstration here of survival in a dystrophic, allogeneic environment for at least 2 months, indicates the ultimate immunological success of progenitor cell transplantation to the diseased central nervous system.

Neuroprogenitor cells like AHPCs are capable of reaching all layers of the retina, and differentiating into cells with local phenotypic characteristics. These cells represent an exciting new tool for studying and manipulating retinal development in mammalian species.

Since neural progenitor cells can be propagated in vitro and, following transplantation, can extensively repopulate an actively degenerating retina in visually mature animals, they will also be useful in treating retinal diseases involving neuronal cell loss. In view of the results discussed herein, it is reasonable to expect that AHPCs and other neural progenitor cells would similarly be able to differentiate into the appropriate neuronal cell lineage of other neural sites into which these progenitors are transplanted *in vivo*. Therefore, AHPC transplantation can be useful also to treat other neurological diseases and injuries involving neuronal loss or damage.

Example II: Xenogeneic Retinal Transplants

The survival of adult rat-derived, hippocampal neural progenitor cells transplanted into the dystrophic mouse retina was investigated. These transplanted cells were capable of integrating into the murine host retina and of maintaining expression of the green fluorescence protein (GFP) gene inserted into the progenitor cells.

Methodology

Neural progenitor cells, cultured from the hippocampus of adult Fischer 344 rats, were genetically modified to express GFP and a clonal cell line was isolated, as previously described. These cells were then transplanted into the vitreous of 7-day-old "rd-1" mice (50,000 cells in 1 µl), without immunosuppression. After 2-4 weeks post-transplant, the eyes were removed and sectioned.

Results and Discussion

At both survival times (2 weeks and 4 weeks post-transplant), large numbers of GFP^+ cells were found in the vitreous of host mice. Many cells were adherent to the inner surface of the retina, where they extended long, axon-like processes. In some cases, cells were found to have migrated into the host retina, where they developed neuron-like phenotypes, and extended numerous processes into the host neuropil.

Rat, adult neural progenitor cells transplanted to a xenogeneic environment without immunosuppression are capable of surviving for at least 4 weeks and maintaining expression of a GFP marker. These cells can also migrate into the host retina, where they developed neuron-like phenotypes. The use of xenogeneic, pluripotent progenitor cells as a source of donor tissue in transplantation protocols offers a viable new technology for studying and manipulating neural development and neural tissue plasticity, and repairing damaged central nervous system (CNS) tissue. In the case of human disease, the present technology will enable the use of xenogenic, neural tissue, such as pig-derived neural progenitor cells, to treat retinal and other neurological diseases and injuries involving neuronal loss.

Example III: Physiological improvement in rats receiving neural progenitor cell transplants

Neuroprogenitor cells such as AHPCs have the capacity to restore vision in blind rodents. Recent experiments have demonstrated that grafts of AHPCs into the eye of RCS rats leads to behavioral recovery, as measured by a optokinetic nystagmas (OKN) reflex test. OKN is an involuntary reflex, which depends upon visual acuity level to generate a response to rotating contrast

gratings. These gratings can be varied by intensity, contrast, and frequency to precisely determine visual acuity. Animals grafted with AHPCs can possess an OKN response, whereas control animals do not. Two possible mechanisms can explain this visual behavior:

1) Grafted AHPCs are actively integrated in the retinal cytoarcitecture, and are contributing in some manner to the visual pathway, either as photoreceptors, interneurons, and/or retinal ganglion cells; and/or

2) Grafted AHPCs lead to rescue of host photoreceptors. If the latter is true, AHPC grafting has great promise in the growing field of growth factor delivery, as grafted cells can integrate into the host retina in a stable manner, and can be modified in vitro to secrete specific molecules into the host retina.

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Other uses of multipotent neural stem/progenitor cells for cell replacement in different disorders.

AHPCs have been grafted in adult rat hippocampus where they migrate and differentiate into neurons in the dentate gyrus (Gage et al., 1995). The site specific migration and integration of these cells have been tested by grafting them in the rostral migratory pathway leading to the olfactory bulb (Suhonen et al., 1997). Cells migrated along the RMP and then laterally to granule cell and gromular cell layers. Cells migrating to the granule cell layer became calbindin/NeuN$^+$ cells. and those migrating to gromeruli became tyrosine hydroxylase$^+$ neurons (typical phenotypes of cells residing in these regions). Similarly, clonal populations of AHPCs grafted in neonate eyes migrated to different layers assuming the morphological characteristics of cells present at those layers. However, they did not express any of the markers specific for eye cells (Takahashi et al., 1998). In addition, adult spinal cord-derived progenitor cells, when grafted in the spinal cord, have been found to generate only glial cells. In contrast, in the hippocampus, they migrate in a similar fashion to AHPCs and differentiate into neurons only in the dentate gyres (Shiabuddin, Horner, Ray and Gage, unpublished results). These results indicate that AHPCs are plastic and that their ultimate fate has been guided by the external stimuli present in a specific region of the organ and not by their internal programming. These observations suggest that these cells can be used for grafting in organs very different from their site of origin. This hypothesis has recently been confirmed by a report showing that stem/progenitor cells derived from adult

mouse brain when transplanted into irradiated mice produce a variety of blood cell types (Bjornson et al., 1999). Two recent reports have shown that fetal human brain-derived neural stem cells grafted into embryonic rats or new born mice participate in aspects of normal development. Grafted cells migrate, incorporate into all major compartments of the brain, and differentiate into multiple developmentally and regionally appropriate cell types (Flax et al., 1998, Brustle et al., 1998). These data indicate that xenografts of multipotent neural stem/progenitor cells not only survive, they behave like endogenous cells of the recipient species. The ultimate fate of the grafted cells is determined by the endogenous stimuli present in specific brain regions.

Grafting of multipotent neural stem/progenitor cells in various organs for reconstitution in various diseases and disorders.

The plastic and pluripotent nature of multipotent neural stem/progenitor cells derived from rat, mouse and human have made them ideal candidates for their use as a source of cells which can be used to replace or correct for cells lost in disease or injury. The utility of these cells for transplantation can be tested in the following disease models.

Liver diseases: The liver plays a central role in the pathophysiology of many inherited metabolic diseases. Despite the unusual ability of the adult liver to regenerate after injury, the liver is an important target for cell therapy. Two separate transgenic mouse models have been established wherein the animals produce a toxic by-product that damages or kills hepatocytes. In albumin-urokinase (Alb-uPA) transgenic mice,

hepatocyte-targeted expression of a hepatotoxic transgene creates a functional liver deficit leading to chronic stimulus of liver growth (Rhim et al., 1994). In a second murine model for human hereditary liver disease, tyrosinaema type I (HTI), a recessive liver disease, is caused by deficiency of fumarylacetoacetate hydroxylase (FAH). Transplantation of hepatocytes from normal animals to spleens of adult transgenic animals showed that the transplanted cells can repopulate 80-90% of the diseased livers. We propose to transplant adult rat hippocampal-derived progenitor cells (AHPCs) or adult mouse-brain derived progenitor cells (AMPCs), or fetal or adult human brain-derived progenitor cells, in to these animal models to determine whether brain-derived progenitor cells can respond to local environment of the spleen, become hepatocytes, and replace the dying cells to correct the disease phenotypes.

Diabetes: Recent experimental data from immune and endocrine studies using spontaneous or transgenic models of the disease have emphasized the role of the islet of Langerhans, and particularly beta cells, in autoimmune insulin-dependent (Type 1) diabetes mellitus (IDDM) pathogenesis. IDDM is a chronic disorder that results from the destruction of the insulin-producing beta cells of the pancreatic islets. In its initial phase, T lymphocytes and other inflammatory cells invade the islets, eventually destroying them. The pathological consequence is the inability of the animals to maintain glucose homeostasis. Most work has focused on the spontaneous model of the disease, the non-obese diabetic (NOD) mouse, which in addition to providing genetic data, appears to be useful for sequential study of the early developmental, immune and endocrine events that occur in IDDM pathophysiology. A transgenic line

overexpressing a T-cell receptor (TCR) that recognizes a natural autoantigen recognized in IDDM has been developed. Viable islet cells isolated from pancreas have been transplanted, resulting in complete reversal of hypoglycaemia in diabetic animals (Thomas et al., 1990). AHPCs, AMPCs, or fetal or adult human brain-derived progenitor cells can be grafted in the pancreas of the NOD or transgenic mice to determine whether hypoglycemia can be corrected by the replacement of the damaged cells with the grafted cells.

Muscle disorders: Duchenne muscular dystrophy (DMD) is characterized by slow and progressive muscle weakness affecting limb and respiratory muscles, which degenerate until fatal cardiorespiratory failure. Myodystrophy of the Duchenne type results from mutations affecting the gene for dystrophin, a cytoskeletal protein. Several types of mutations have been described, which encompass the complete absence of dystrophin to its presence in reduced levels or the presence of partially functional truncated forms, and which lead to severe to very mild forms of the disease (Gilis, 1996). The mdx mice that showed complete absence of dystrophin have been used as a model for DMD and have been tested for cell therapy. Normal myoblasts have also been transplanted into the muscle of patients with DMD. A form of congenital dystrophy caused by a deficiency of the a2 subunit of the basement membrane protein laminin/merosin is termed merosin-deficient congenital muscular dystrophy (MCMD). Most patients with MCMD are never able to walk. Null mutant dyw mice have been generated (Kung et al., 1998). Expression of human LAMA2 gene in the skeletal muscle of dyw mice dramatically improves the muscle disease in these animals. Both mdx mice and dyw mice can be used for transplantation of AHPCs, AMPCs or fetal or adult human brain-derived progenitor cells into muscles,

to determine whether these cells can become myoblasts and replace degenerating muscle cells.

Cardiovascular disease: The regulation of cardiovascular function is complex and depends on many factors interacting in a defined and temporal fashion. Knock-out mice lacking desmin, needed to maintain the integrity of the myocardium to develop cariomyopathy (Lie et al., 1996; Milner et al., 1996, Thornell et al., 1997). AHPCs, AMPCs, or fetal or adult human brain-derived progenitor cells can be grafted in the myocardium of these mice to determine whether the transplanted cells can replace the diseased cells and improve heart function.

Pulmonary disease (Cystic fibrosis): Cystic fibrosis, the most common autosomally inherited disease, is caused by the defective gene Cftr, which encodes an ion channel at the cell membrane. By homologeous recombination, several groups have disrupted the Cftr gene. All null mutation mice developed symptoms of cystic fibrosis (Dorin et al., 1992). AHPCs, AMPCs, or fetal or adult human brain-derived progenitor cells can be grafted into the lung of these mutant mice to determine whether these cells can replace the diseased cells having defective ion channels, and restore normal lung function.

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CLAIMS

What is claimed is:

1. A method of treating dystrophic neural tissue, comprising introducing neural progenitor cells derived from an adult animal donor into dystrophic neural tissue in an animal recipient.

2. A method of repopulating or rescuing a dystrophic retina or optic nerve with neural cells, comprising introducing neural progenitor cells derived from an adult donor into dystrophic retinal or optic nerve tissue in an animal recipient.

3. The method of claim 1, wherein said neural progenitor cells are introduced into the recipient's central nervous system (CNS).

4. The method of claim 1 or 2, wherein said neural progenitor cells are placed in a site selected from the group consisting of an eye, an optic nerve, and a vitreous.

5. The method of claim 1 or 2, wherein said neural progenitor cells are clonally derived.

6. The method of claim 1 or 2, wherein said neural progenitor cells are derived from brain tissue.

7. The method of claim 1 or 2, wherein said neural progenitor cells are derived from a hippocampus or a ventricular zone.

8. The method of claim 1 or 2, wherein said recipient is an immature or young animal.

9. The method of claim 1 or 2, wherein said recipient is an adult.

10. The method of claim 1 or 2, wherein said recipient is a human.

11. The method of claim 1 or 2, wherein said donor and said recipient are of different species.

12. The method of claim 11, wherein said donor and recipient pair is selected from the group consisting of the following pairs: a rat donor and a mouse recipient; a mouse donor and a rat recipient; a pig donor and a human recipient.

13. The method of claim 1 or 2, wherein said donor and said recipient are of the same species.

14. The method of claim 13, wherein said donor and said recipient are allogeneic.

15. The method of claim 13, wherein said donor and said recipient are syngeneic.

16. The method of claim 2, wherein said dystrophic retinal tissue is a result of an optic neuropathy.

17. The method of claim 2, wherein said dystrophic retinal tissue is a result of glaucoma.

18. The method of claim 1 or 2, wherein said neural progenitor cells have been cultured in vitro in a culture medium comprising at least one trophic factor.

19. The method of claim 18, wherein the at least one trophic factor is selected from the group consisting of a neural growth factor; a neurotrophin; a mitogen; a cytokine; a growth factor; a hormone; and a combination thereof.

20. The method of claim 18, wherein said culture medium comprises a member selected from the group consisting of: fibroblast growth factor alone; fibroblast growth factor and epidermal growth factor; and fibroblast growth factor and epidermal growth factor and heparin.

21. The method of claim 1 or 2, wherein said neural progenitor cells have been derived by performing the steps of:

(a) isolating fresh neural progenitor cells from an adult donor animal;

(b) culturing said freshly isolated neural progenitor cells on a polyornithene/laminin-coated substrate, in a culture medium comprising at least one trophic factor;

(c) incorporating an identifying, genetic marker into said cultured progenitor cells; and

(d) cloning individual neural progenitor cell lines from the cultured cells resulting from step (c).

22. The method of claim 20, wherein the at least one trophic factor is selected from the group consisting of a neural growth factor; a neurotrophin; a mitogen; a

cytokine; a growth factor; a hormone; and a combination thereof.

23. The method of claim 20, wherein said neural progenitor cells are derived from brain tissue.

24. The method of claim 20, wherein the neural progenitor cells are derived from a hippocampus or a ventricular zone.

25. The method of claim 5, further comprising, prior to introducing said neural progenitor cells into an animal recipient, confirming the lineage potential of each clone of neural progenitor cells by inducing a sample of said clonally derived neural progenitor cells to differentiate in conditioned medium.

FIG. 1a

FIG. 1b

FIG. 1c

FIG. 1d

FIG. 1e

FIG. 1f

FIG. 2a *FIG. 2b* *FIG. 2c*

FIG. 2d *FIG. 2e* *FIG. 2f*

FIG. 2g *FIG. 2h* *FIG. 2i*

FIG. 3a

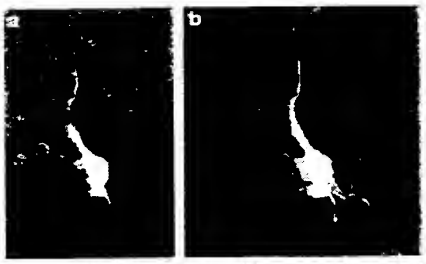

FIG. 3b

FIG. 3c

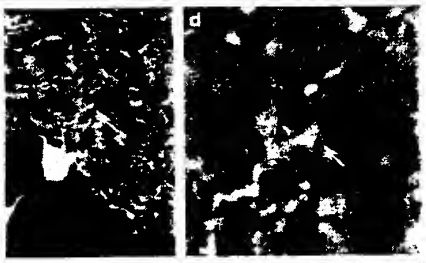

FIG. 3d

FIG. 3e

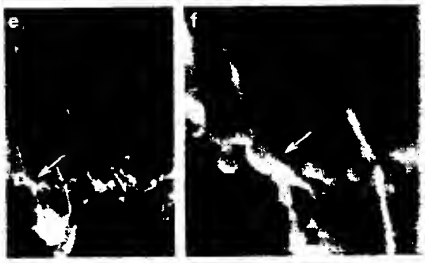

FIG. 3f

FIG. 3g

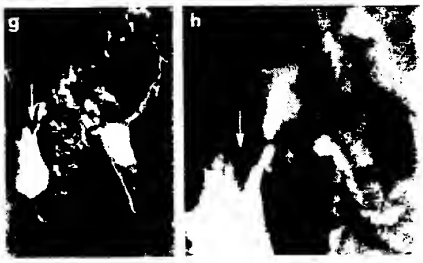

FIG. 3h

FIG. 4a *FIG. 4b*

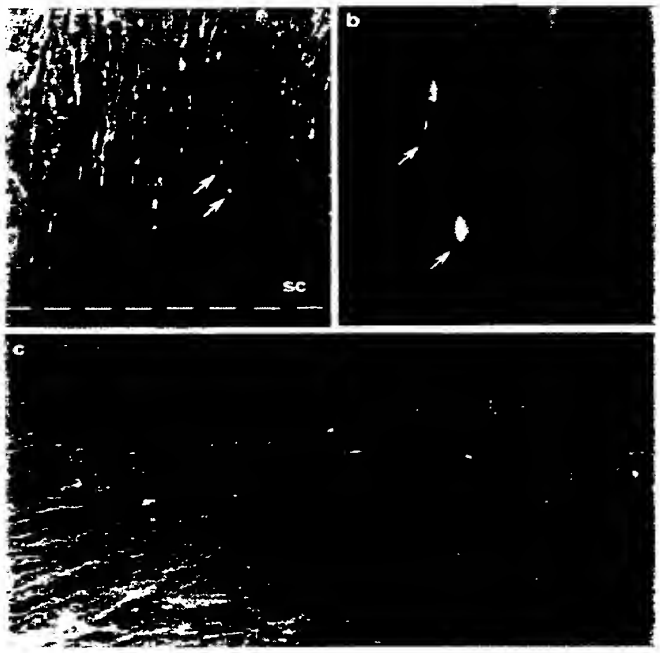

FIG. 4c

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03534

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 48/00; C12N 15/85; A01N63/00
US CL : 424/93.21, 93.7, 93.1; 435/405, 325

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.21, 93.1, 93.7; 435/405, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN (medline, biosis, caplus, scisearch, embase), WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTINEZ-SERRANO et al. Immortalized neural progenitor cells for CNS gene transfer and repair. Trends in Neuroscience. November 1997, Vol. 20, No. 11, pages 530-538, see abstract and page 530, paragraph 4.	1, 3, 4-15, 18-25.
X	SNYDER et al. Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. Nature. 23 March 1995, Vol. 374, page 367-370, see abstract and page 367, paragraphs 1-5.	1,3,4-15,18-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
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Date of the actual completion of the international search 13 APRIL 2000	Date of mailing of the international search report 30 MAY 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID NIKODEM Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/03534

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TAKAHASHI et al. Widespread integration and survival of adult-derived neural progenitor cells in the developing optic retina. Molecular and Cellular Neuroscience. December 1998, Vol. 12, pages 340-348, see abstract and page 346, paragraph 2.	2,4-15,16-2 5
X	MARTINEZ-SERRANO et al. CNS-derived neural progenitor cells for gene transfer of nerve growth factor to the adult rat brain: complete rescue of axotomized cholinergic neurons after transplantation into the septum. The Journal of Neuroscience. August 1995, Vol. 15, No. 8, pages 5668-5680, see abstract and page 5668, paragraph 1-2.	1, 3, 4-15,18-25
Y	DURING et al. Towards gene therapy for the central nervous system (review). Molecular Medicine Today. November 1998, Vol. 4, No. 11, pages 485-493, see page 485, paragraphs 3-6.	1-25.